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Targeting PI3K Isoforms and SHIP in the Immune System: New Therapeutics for Inflammation and Leukemia

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Targeting PI3K signaling in the immune system

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Abstract:

PI3K is critical for the normal function of the immune system, however dysregulated PI3K mediated signalling has been linked to the development of many immune mediated pathologies. This review describes current progress in the development of isoform-specific PI3K inhibitors that hold promise for the treatment of hematopoietic malignancies as well as for inflammatory and autoimmune diseases. A SH2-domain containing inositol-5-phosphatase (SHIP) is a regulator of PI3K signalling, and is also discussed as a potential drug target for immunomodulation and the treatment of leukemia. Recent progress has been made in the development of small molecule compounds that potently and selectively modulate SHIP activity and hence provide a novel mechanism to alter PI3K mediated signalling.

Introduction

Activation of class 1 phosphoinositide 3-kinase (PI3K) occurs following ligation of a plethora of leukocyte receptors that are responsible for innate and adaptive immune responses [1-3]. Activation of PI3K results in the addition of a phosphate group to its lipid substrate PI(4,5)P₂, forming PI(3,4,5)P₃ in the cell surface membrane. PI(3,4,5)P₃ initiates a variety of signalling cascades by interaction with pleckstrin homology (PH) domain containing proteins, most notably the serine kinase Akt [4].

Class 1A isoforms (PI3K α , PI3K β and PI3K δ) are activated by receptor tyrosine kinases, whilst the Class 1B isoform PI3K γ is activated following GPCR ligation. However, these definitions are proving less rigid than previously thought. For example, PI3K β has been shown to signal downstream of GPCR activation [5], whilst activation of the Class 1B isoform PI3K γ has been reported by receptor tyrosine kinases and TLR/IL-1Rs [6]. PI3K γ has also been reported to participate in T-cell-receptor signalling [7]. The PI3K α and PI3K β isoforms appear to have a broad tissue distribution, while PI3K δ and PI3K γ are primarily expressed in leukocytes. Studies using mice in which the genes encoding PI3K δ or PI3K γ have been either altered to encode kinase-inactive mutants (e.g. PI3K δ^{D910A} mice) or deleted, have revealed that PI3K δ and PI3K γ have non-redundant but often co-ordinated, functions in B cells, T cells, NK cell, neutrophils, mast cells and dendritic cells [1-3,8,9]. Indeed, when the immune system of these mice is challenged they exhibit severely defective responses to infection [1-3].

Development of PI3K inhibitors to target the immune system: lessons from PI3K δ

The strong evidence of a role of dysregulated PI3K γ and PI3K δ in immune-mediated pathologies, has prompted intense efforts to develop selective inhibitors of these isoforms. The first compounds to block PI3K—the natural product wortmannin and Lilly's Ly294002 were reported in the early 1990's and served as useful experimental tools to research the PI3K pathway [2]. This is evidenced by nearly 6000 PubMed hits for Ly294002 alone. However, the usefulness of wortmannin and Ly294002 both as research tools and potential drugs was tempered by their broad targeting of all PI3K isoforms and off-target effects [10]. However, the simple planar structure of Ly294002 (**Figure 1**) has aided the design of more selective pan-PI3K and isoform specific inhibitors. In particular, crystal structures of PI3Ks bound to Ly294002, have facilitated huge advances in the design of PI3K inhibitors which utilize the regions of the ATP binding pocket to achieve greater potency and selectivity as well as reduced toxicity [11-13]

Compounds that selectively inhibit PI3K γ have been identified, with a series of compounds designed by Merck Serono SA based on the thiazolidinedione scaffold. One of these, AS-605240 (**Figure 1**), exhibited superior potency compared to related compounds, can be administered orally and has high cell membrane permeability [14]. However, the subsequent development of PI3K γ inhibitors for the treatment of inflammatory disorders in humans has been largely disappointing. This is most likely due to the selectivity achieved for PI3K γ over other class 1A PI3K isoforms, being insufficient to avoid off-target effects. In contrast, the development and implementation of inhibitors which target the PI3K δ isoform has proven more successful. The discovery of the quinazolinone purine series, exemplified by the ICOS compound IC-87114 (**Figure 1**) demonstrated that the design of isoform selective PI3K inhibitors with at least 50-fold potency over other isoforms was possible to achieve [15].

In 2006 several members of ICOS Corporation formed a spin-out company, Calistoga Pharmaceuticals. Calistoga developed CAL-101, a PI3K δ specific inhibitor that exhibits 40-300 fold selectivity over other PI3K isoforms. CAL-101 which was acquired by Gilead in February 2011 and recently renamed GS-1101) has shown success in clinical trials for treatment of B cell malignancies where it causes rapid lymph node shrinkage and lymphocytosis. CAL-101 displays a dual mechanism of action whereby it both decreases cell survival and reduces interactions that retain CLL cells in protective tissue microenvironments [16,17]. This inhibitor has therefore demonstrated an essential role for PI3K δ in constitutive PI3K signaling that is required for the survival of malignant B cells. Oncogenic mutations of components of the PI3K signaling pathway are infrequent in B cell malignancies. A potential mechanism for PI3K activation in this setting is tonic antigen-independent B cell receptor (BCR) signaling that requires PI3K δ for the transduction of proliferation and survival signals.

Inhibition of the PI3K δ isoform for the treatment of inflammatory disorders is also being explored. Specifically, CAL-101 and CAL-263 have entered clinical trials for allergic rhinitis (**Table 1**). In addition, patents have been filed by several other companies (Amgen, Intellikine and Incyte) describing PI3K δ inhibitors and the majority are based on the same basic pharmacophore identified by ICOS [18]. However, additional scaffolds have now been reported by several companies; almost all of these are with intended indications against B cell lymphomas [18].

The non-redundant and often coordinated roles of PI3K δ and PI3K γ in immune cell function described earlier, provide a rationale for targeting both isoforms simultaneously with a single compound. TargeGen described two diaminopteridine-diphenol-based compounds with good selectivity for PI3K γ and PI3K δ that showed early promise in animal models of myocardial ischemia as well as asthma and chronic obstructive pulmonary disease [19,20]. The TargeGen

compounds did not progress beyond phase I/II clinical trials. Infinity and Intellikine are currently in pre-clinical trials with IPI-145 (**Table 1**), the only PI3K γ/δ inhibitor currently in development for the treatment of inflammatory disease [18].

There is an increasing appreciation of a role for PI3K β in the immune system including cooperation with PI3K δ in the generation of reactive oxygen species (ROS) in neutrophils in response to fungal infection or immune complexes [21,22]. This provides a rationale for targeting PI3K β as well as PI3K δ in the treatment of inflammatory disorders. Indeed, compounds with dual selectivity for PI3K β and PI3K δ have been reported suggesting that this approach is feasible [13]. However, caution should be applied to the use of PI3K β inhibitors in inflammatory disorders due to the described role of PI3K β in thrombus formation and circulatory homeostasis [23].

SHIP-1 as an alternative target for modulation of PI3K signaling.

Role of SHIP in the immune system

The development of isoform specific PI3K inhibitors for inflammatory disease, particularly toward PI3K γ , has been disappointing. This has led to the search for alternative targets with which to modulate PI3K signalling specifically in the immune system. In this regard, attention has recently focussed on the lipid phosphatase SH2-domain containing inositol-5-phosphatase (SHIP), which de-phosphorylates PI(3,4,5)P₃ at the D5 position of the inositol ring to create PI(3,4)P₂. Multiple forms of SHIP have been reported, but the 145 kDa SHIP-1 (**Figure 2**) is a particularly ideal target for development of potential therapeutics for treating immune disorders. This is because its hematopoietic-restricted expression would limit the impact of SHIP-1 targeted drugs to the immune system. One would predict for example, that activators of SHIP-1 would lead to a reduction of cellular PI(3,4,5)P₃ levels and hence, mimic the effect

of PI3K inhibitors. SHIP-1 was initially considered as a negative regulator of PI3K signalling. However, the SHIP-1 product PI(3,4)P₂ is itself recognized by some PH domain-containing proteins [24]. Thus SHIP-1 can act as a molecular ‘switch’, re-directing PI3K signalling away from PI(3,4,5)P₃-dependent effector proteins, towards proteins which bind exclusively or partially to PI(3,4)P₂.

SHIP-1 is recruited to the surface membrane following ligation of a variety of receptors including, chemokine, antigen, co-stimulatory and cytokine receptors as well as IgG engagement [25]. SHIP-1 knockout mice have proven invaluable in identifying the crucial role of SHIP-1 in the regulation of mast cell degranulation, BCR signaling and autoantibody production, dendritic cell function and NK cell cytolytic function (**Table 2**). SHIP-1 also regulates TLR signaling [26,27,28], lymphocyte polarization and migration [29] and has a pivotal role in regulating the balance between pro-inflammatory and anti-inflammatory myeloid and lymphoid cells [30,31].

The Role of SHIP-1 in leukemia

Over-activation of PI3K-dependent signalling cascades is a common occurrence in many human cancers [32]. The lipid phosphatase PTEN which also negatively regulates PI(3,4,5)P₃ accumulation by de-phosphorylating the D3 position of the inositol ring, is a well characterized tumour suppressor gene [33]. Likewise, evidence for mutations of SHIP-1 have also been shown in acute lymphoblastic leukaemia [34] and in acute myeloid leukaemia [35]. SHIP-1 is targeted by miR-155 in B cells, with high levels of miR-155 and reduced SHIP-1 expression linked to the development of acute lymphoblastic leukaemia in mice [36]. miR-155 levels were also found to be significantly increased in human patients with diffuse large B cell lymphoma [37]. The loss of SHIP-1 has been shown to promote the development of erythroleukemia, with SHIP-1 identified as a target gene of the oncogene fli-1 [38]. The loss

of SHIP-1 has been shown to promote the development of erythroleukemia, with SHIP-1 identified as a target gene of the oncogene *fli-1* [38]. The role of SHIP-1 as a tumour suppressor is also evident in the ability of SHIP-1 to restrict myeloid suppressor cells and regulatory T cells [30,39]. Therefore the loss of SHIP-1 expression/ function may lead to increased suppression of T-cell mediated anti-tumour immunity. Indeed, in murine pancreatic cancer SHIP-1 expression was shown to be reduced in splenocytes which also correlated with an increase in myeloid suppressor cell numbers [40]. Decreased SHIP-1 expression has also been shown in myelodysplastic syndrome progenitor cells, where over-expression of SHIP-1 inhibited myeloid leukemic growth [41].

The role of SHIP-1 in leukemia however, appears more complex than initially thought. For example, SHIP-1 was shown not to act as a tumour suppressor in myeloma cells [42]. The use of a small molecule SHIP-1 inhibitor demonstrated that catalytically active SHIP-1 is required for the survival of multiple myeloma cells [43] and that therefore, in certain cases, SHIP-1 actually supports cancer cell survival. This would be consistent with increased levels of the SHIP-1 enzymatic product $PI(3,4)P_2$ promoting Akt activation and survival/proliferation [44]. Indeed another group has shown that SHIP-1 inhibits CD95/Fas-mediated apoptosis of T cells [45].

Pharmacological Targeting of SHIP

Allosteric SHIP-1 activators:

The identification and description of an activator of SHIP-1, termed pelorol, was first described in 2005 and was derived from the marine invertebrate *Dactylospongia elegans* [46]. Aquinox Pharmaceuticals designed more potent synthetic analogies AQX-MN100 and AQX-016A (**Figure 1**) which along with $PI(3,4)P_2$, were shown to allosterically enhance catalytic

activity by binding to the C2 domain of SHIP-1 (**Figure 2**). AQX-016A and AQX-MN100 showed potent inhibition of immune cell activation *in vitro* and were anti-inflammatory *in vivo* using mouse models of endotoxemia and acute cutaneous anaphylaxis [47]. Intriguingly, these SHIP-1 activating compounds increased apoptosis of multiple myeloma cells *in vitro* and when used in combination with bortezomib (an established multiple myeloma treatment) proved more effective at inhibiting cancer cell proliferation than bortezomib alone [48]. Aquinox Pharmaceuticals are developing SHIP-1 activating compounds based on the structure of pelorol, to be used in inflammatory disorders. AQX-1125 is the most advanced and passed Phase 1 clinical trials in 2011, with Phase IIa clinical studies initiated in late 2011 for the treatment of mild and moderate asthma (**Table 1**)

SHIP-1 inhibitors

A novel small molecule selective inhibitor of SHIP-1, termed 3 α -aminocholestane (3AC, **Figure 1**) has also recently been identified using high throughput screening, although the site of action is currently unclear [43]. Consistent with observations from SHIP-1 deficient mice, treatment of mice with 3AC led to increased numbers of myeloid suppressor cells and reduced ability of peripheral lymphoid tissues to prime myeloid-associated responses and protected against Graft-versus-host disease [43]. The inhibition of SHIP-1 using pharmacological compounds may therefore offer the potential to aid transplant acceptance in patients undergoing transplant surgery. 3AC also increased levels of granulocytes, red blood cells, neutrophils and platelets in mice and could therefore, have potential to improve blood cell number in patients with myelodysplastic syndrome or myelosuppressive infection.

Remarkably, SHIP-1 inhibition using 3AC induced the apoptosis of human AML cell lines which is consistent with SHIP-1 being anti-apoptotic under some circumstances [43]. Further studies showed that 3AC inhibited multiple myeloma cell growth in a tumour xenograft

model in mice [49]. Since both substrate [PI(3,4,5)P₃] and product [PI(3,4)P₂] of SHIP-1 have been shown to influence Akt activation and cell survival, this may explain in part, why both activators and inhibitors of SHIP-1 have shown efficacy against leukemic cells [50].

SHIP-2 inhibitors

SHIP-2 shares 35% homology with SHIP-1, and is thought to be involved in type-2 diabetes and obesity [51]. Its ubiquitous expression means that it is also expressed in leukocytes and one concern is that inhibition of SHIP-1 will be compensated for by SHIP-2. In recent years progress has been made in the development of small molecule compounds which inhibit the catalytic activity of SHIP-2 [52]. Another group has identified a novel biphenyl 2,3',4,5',6-pentakisphosphate [BiPh(2,3',4,5',6)P₅], which showed potent inhibition of SHIP-2 catalytic activity [53]. This biphenyl compound is however, not cell permeable and does not possess drug-like properties. Nevertheless, the crystal structure of the phosphatase domain of SHIP2 bound to BiPh(2,3',4,5',6)P₅ has recently been reported. Molecular dynamics simulations suggest that when BiPh(2,3',4,5',6)P₅ binds to SHIP2, a flexible loop folds over and encloses the ligand [54]. Compounds targeting such a closed conformation might therefore deliver SHIP2-specific drugs. Recently, pan-SHIP-1/2 inhibitors have been reported to kill multiple myeloma cells [49]. The identification of both pan-SHIP and SHIP-2 inhibitors therefore provides a useful tool in the identification of the less well-characterized role of SHIP-2 in immune cell function.

Summary

The β , γ and δ isoforms of PI3K have important non-redundant roles in multiple cells of the immune system. Consequently alterations of the PI3K signaling pathway can lead to inflammatory and autoimmune disorders as well as leukemia. This, together with growing appreciation of the crystal structure of the catalytic isoforms which help define the structure-

activity rules for obtaining selectivity, will spur the continued design and development of improved PI3K inhibitors. These offer opportunities to manipulate the PI3K signaling network in immune cells for inflammation and transplantation as well as cancer. The latter may include non-leukemic cancers, given the up-regulation of PI3K γ and PI3K δ in some forms of non-immune cell malignancies [32,55,56]. One concern would be that it might be difficult to avoid effects on the immune system that could impair the endogenous anti-tumor response.

The difficulties of developing PI3K γ inhibitors with sufficient selectivity over PI3K isoforms has in part, led to the exploitation of endogenous and leukocyte-restricted regulators of PI3K signaling, namely the lipid phosphatase SHIP-1. Small molecule regulators of this protein have shown early promise in inflammatory, transplantation and cancer settings, and are currently in phase I clinical trials to evaluate the safety, tolerability and pharmacokinetics (**Table 1**). However, the targeting of SHIP-1 (particularly with inhibitors), is not without its problems. For example, SHIP-1 deficiency leads to a number of pathologies including fibrotic lung disease [57], osteoporosis [58] and the development of Crohn's disease-like ileitis [59]. Another point to take into account when pharmacologically targeting SHIP-1, is the fact that SHIP-1 has important non-catalytic functions [60]. Therefore inhibition of the catalytic site of SHIP-1 may not be sufficient to inhibit SHIP-1 mediated modulation of PI3K activity in all cases.

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Figure Legends:

Figure 1: Chemical Structures of PI3K inhibitors and SHIP-targeting compounds.

Figure 2. Structure of SHIP-1

SHIP-1 possesses a centrally located 5' phosphatase catalytic domain. A SH2 domain at the N-terminus and NPXY motifs at the C-terminus [25]. SHIP also has a C2 domain adjacent to the catalytic domain which, when bound to PI(3,4)P₂, acts to allosterically enhance the catalytic activity of SHIP [47].

Table 1. Clinical trials status of PI3K and SHIP-1 targeting compounds for the treatment of inflammatory disorders

Compound	Company	Target	Phase	Status	Condition	Reference
CAL-101	Gilead Sciences	PI3K δ	I	Completed	Allergic Rhinitis	http://clinicaltrials.gov/ct2/show/NC T00836914
CAL-263	Gilead Sciences	PI3K δ	I	Completed	Allergic Rhinitis	http://clinicaltrials.gov/ct2/show/NC T01066611
IPI-145	Infinity and Intellikine	PI3K δ/γ	I	Initiated	Inflammatory disorders	http://www.intellikine.com/pipeline/ipi145.html
AQX-1125	Aquinox Pharmaceuticals	SHIP-1	IIa	Initiated	Asthma	http://www.aqxpharma.com/content/aquinox-pharmaceuticals-initiates-two-phase-ii-a-clinical-studies-airway-inflammation

Table 2. Impact of SHIP-1 Gene Targeting on Leukocytes

Cell Type	Phenotype of SHIP-1 KO
Mast cell	Degranulation with IgE stimulation alone [61]
T cell	Increased regulatory T cell differentiation, decreased Th17 development [30] Enhanced Th1 differentiation and CD8 cytotoxic activity. Decreased Th2 differentiation [62]
Basophils	SHIP ^{-/-} mice show increased Th2 skewing due to increased IL-4 secretion from basophils [31])
B cell	Btk membrane association increased. Hyper-responsive to cross-linking of BCR [63] Loss of anergy, production of auto-antibodies [64]
Myeloid cell	Increased myeloid suppressor cell numbers [39] Increased M2 macrophage skewing (indirect mechanism via increased IL-4 secretion from basophils) [65] Increased ratio of PI(3,4,5)P ₃ to PI(3,4)P ₂ on phagosomal membrane. Decreased early NADPH oxidative activity in phagosomes [66]
Dendritic cell	Enhanced survival and proliferation, but impaired maturation [67] Reduced nitric oxide production; SHIP null DC's suppress T cell proliferation [68]
Natural Killer cells	Deficient receptor repertoire. Defective IFN γ secretion. Increase in peripheral number. Defective cytolytic function. [69].

